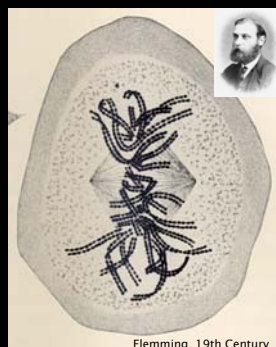


Part III: Mining the Genome for Mitotic Treasures  
An RNAi Screen for Mitotic Spindle Assembly

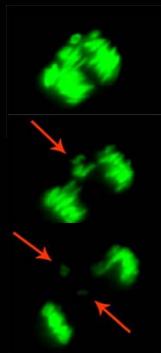
Ron Vale  
UCSF  
HHMI

Movie by Sarah  
Goodwin, UCSF

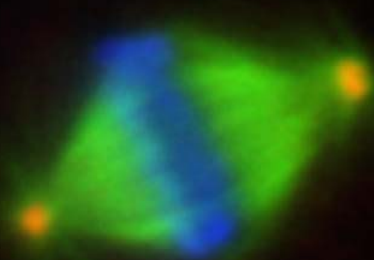
Mitosis is an old problem



and a medically important...  
(aneuploidy in cancer, cancer chemotherapy)



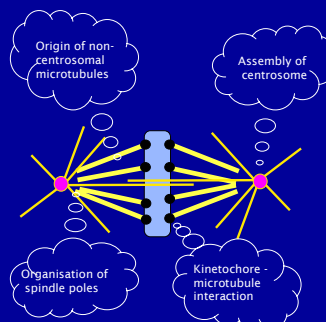
What are the molecules and molecular  
interactions that build the metaphase spindle?

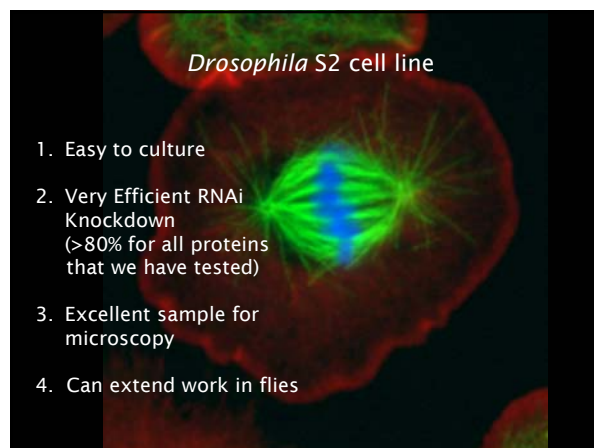
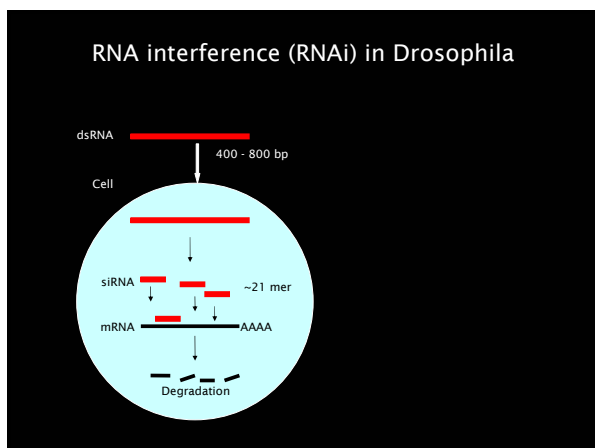
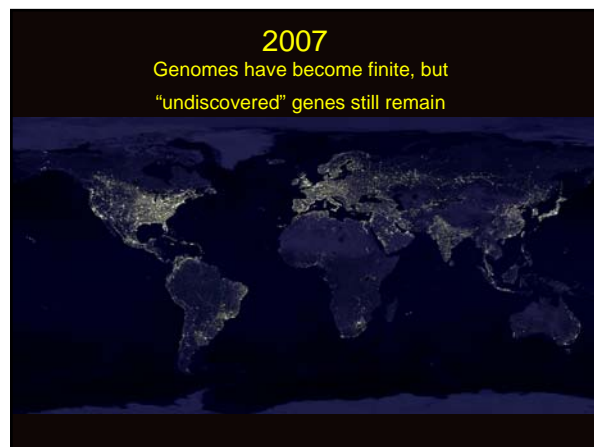
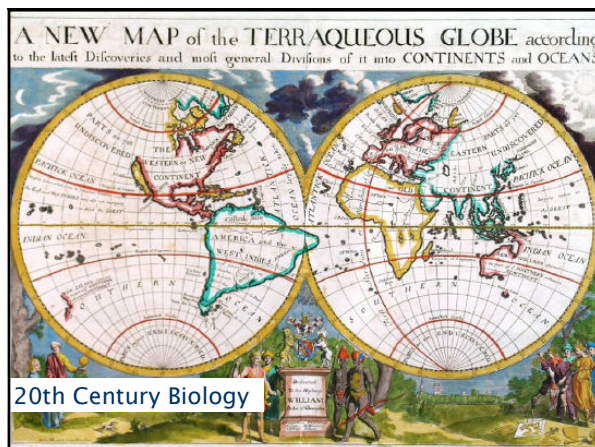
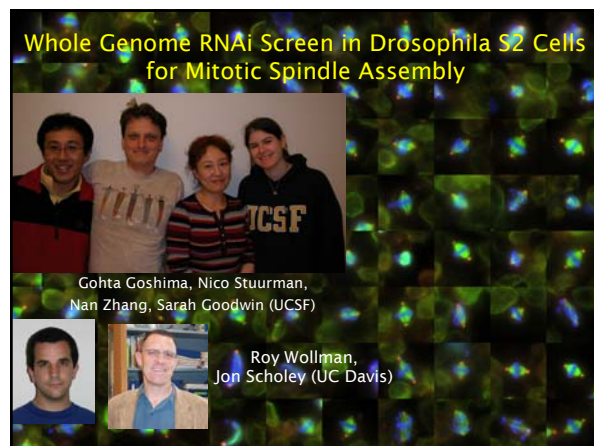
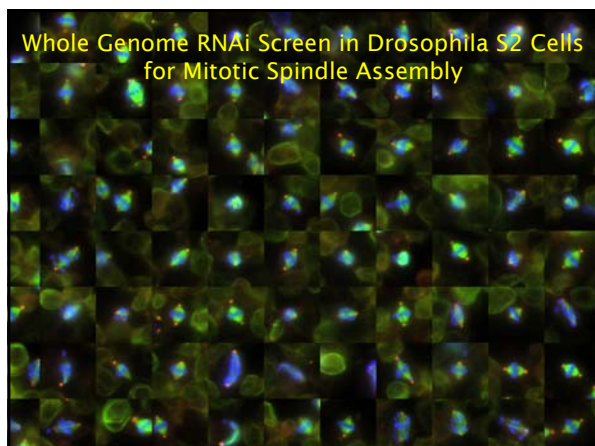


MOVIE

QuickTime™ and a  
MPEG-4 Video decompressor  
are needed to see this picture.

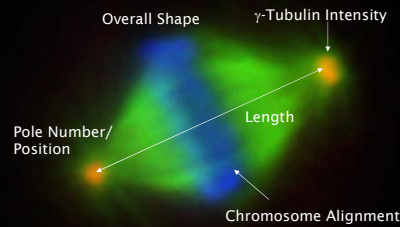
Open questions on spindle morphogenesis





### Image-Based Approach for Identifying Spindle Defects Generated by RNAi

(Goshima et al. Science, April 23, 2007)



14,400 Genes and  
4,000,000 Spindles Analyzed in this Screen

### High-throughput RNAi Screen

#### 1. Full Fly Genome dsRNA Library:

Designed by Nico Stuurman (UCSF)  
(available at Open Biosystems, Inc.)

### High-throughput RNAi Screen

#### 2. Treat S2 Cells with dsRNA for 4 days

96-well, plastic dish x 146  
(each well has dsRNA for one gene)



+ APC dsRNA to  
induce metaphase arrest

### High-throughput RNAi Screen

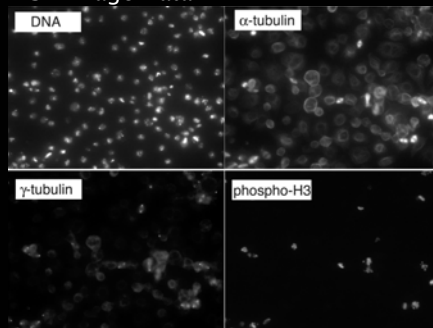
#### 2. High-throughput Microscopy

96-well, glass-bottom dish  
for 40X, 0.95 NA imaging



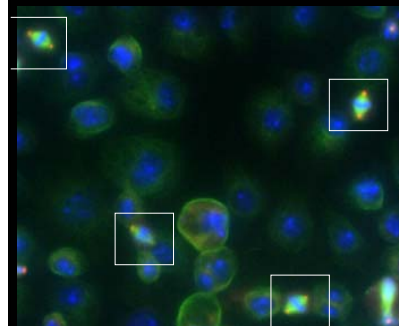
### High-throughput RNAi Screen

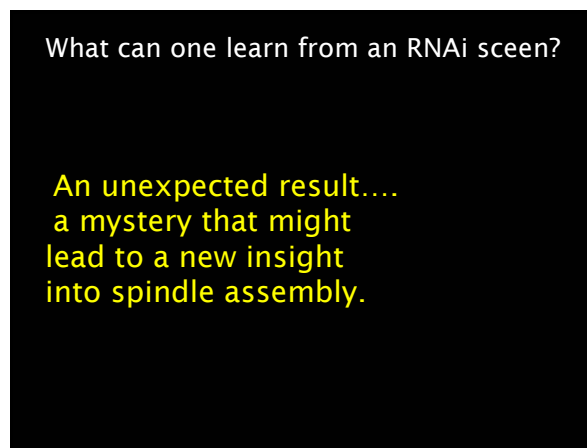
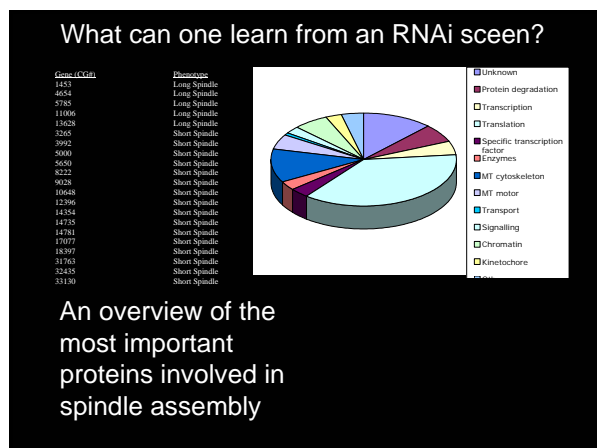
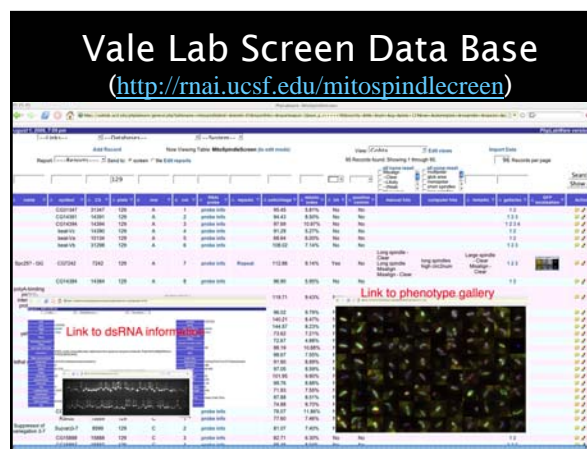
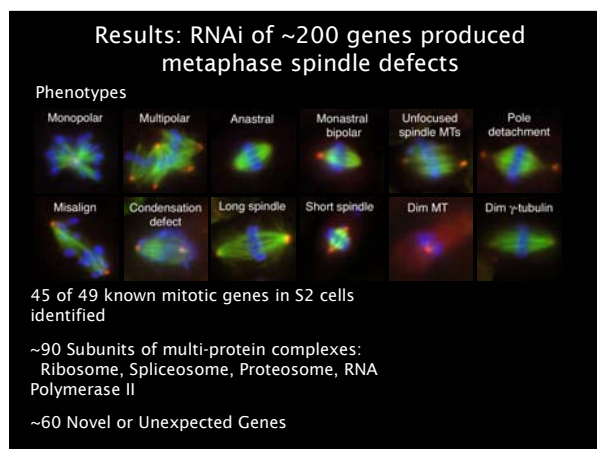
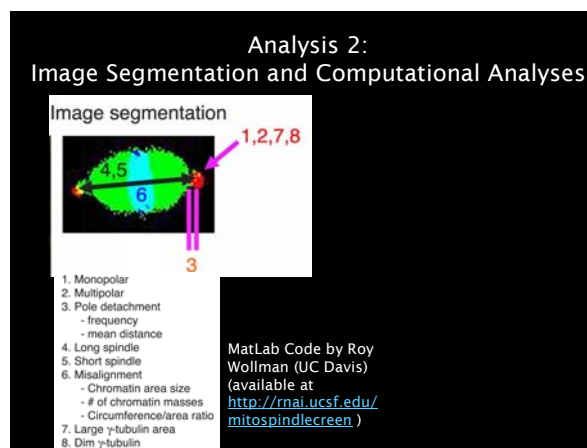
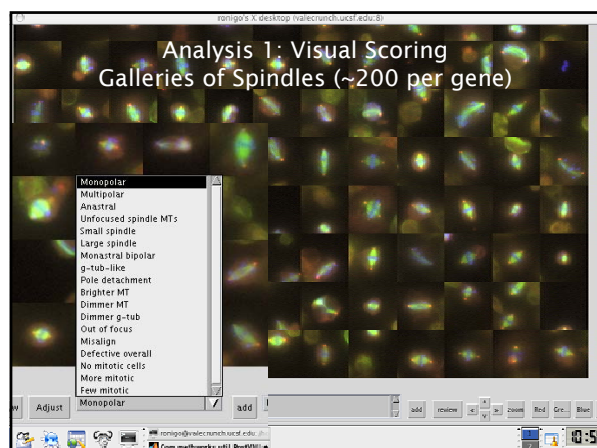
#### 3. Image Data



### High-throughput RNAi Screen

#### 4. Automated Mitotic Cell Identification





### To Track Down These Unexpected Results, a Broad Range of Secondary Assays is Essential!

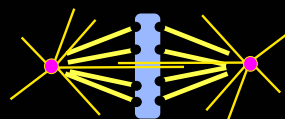
GFP tagging (often N and C termini to be certain of localization results)

Time-lapse imaging of phenotypes

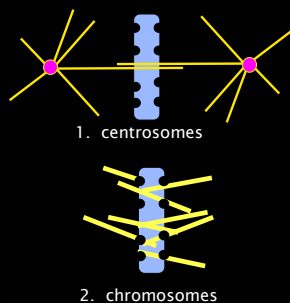
Additional RNAi/drug/localization experiments to understand mechanism

### One story from the screen

#### Making Microtubules to Build the Spindle

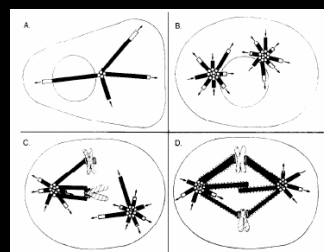


#### Two Main Theories: Microtubule Nucleation by..



#### How are Spindle Microtubules Made?

##### 1. Centrosomal-Nucleated Microtubules



"Search and capture" from the centrosome  
Kirschner and Mitchison, Cell 1986

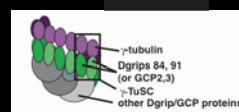
#### How are Spindle Microtubules Made?

##### 1. Centrosomal-Nucleated Microtubules

GFP-tagged EB1, Microtubule plus end tracking protein

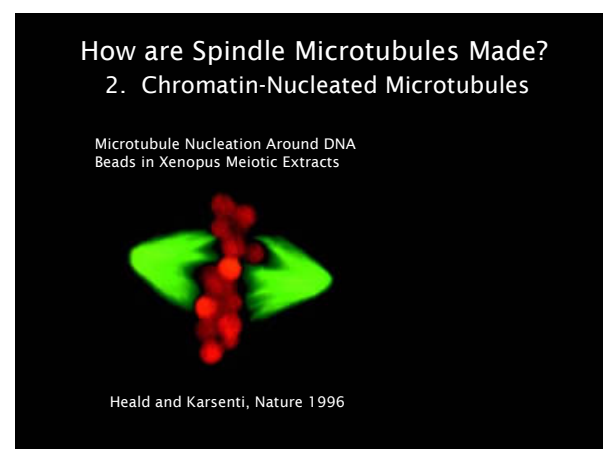
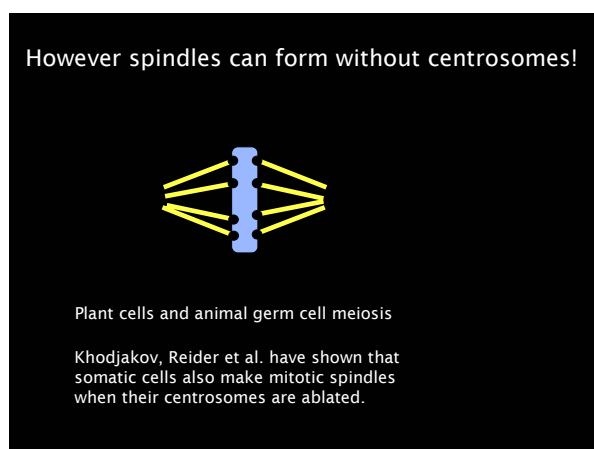
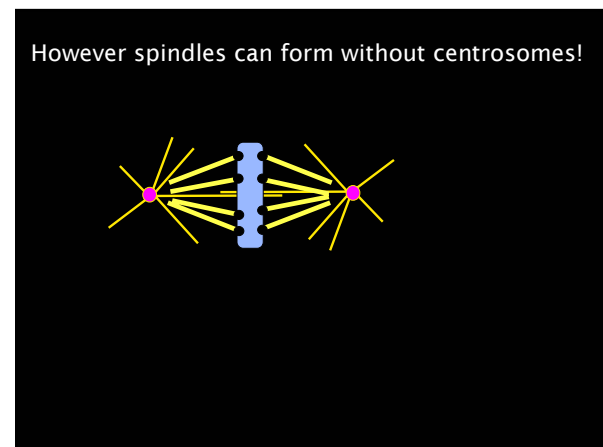
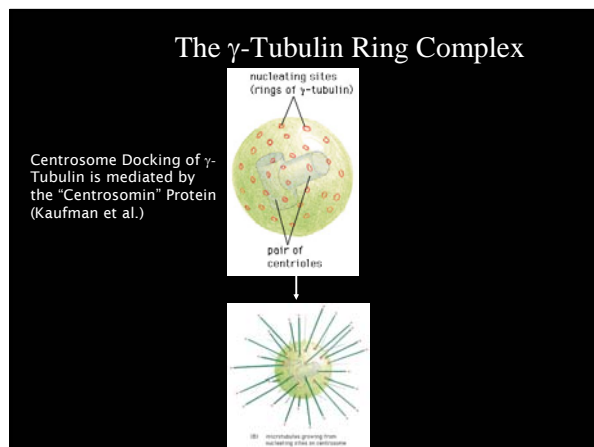
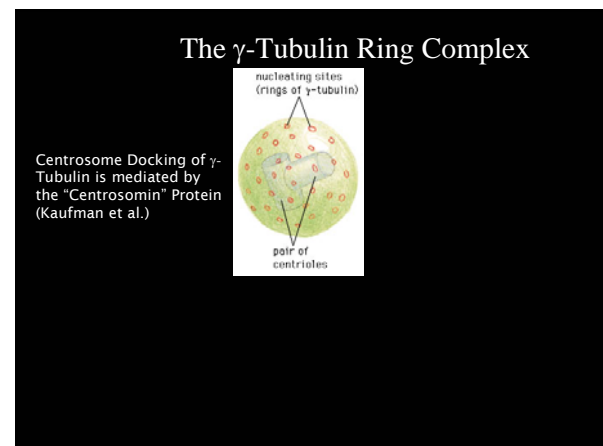
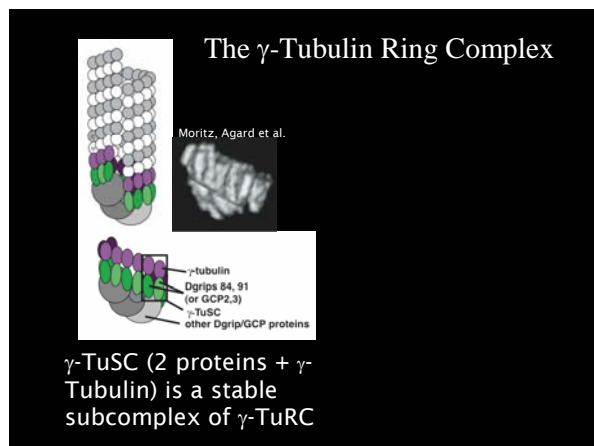
#### The $\gamma$ -Tubulin Ring Complex

Moritz, Agard et al.



$\gamma$ -TuSC (2 proteins +  $\gamma$ -Tubulin) is a stable subcomplex of  $\gamma$ -TuRC





## How are Spindle Microtubules Made? 2. Chromatin-Nucleated Microtubules

Chromatin microtubule nucleation in S2 cells

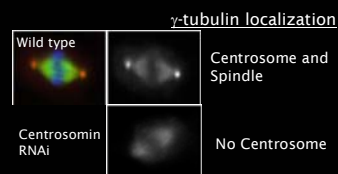
## Microtubule nucleation from a spindle without centrosomes

EB1-GFP with Cnn RNAi  
N. Mahoney et al. *Curr Biol* (2006)

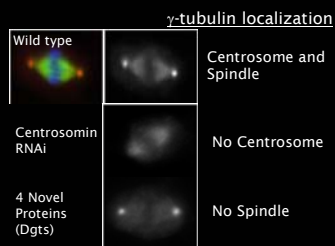
## Dgt (dim $\gamma$ -tubulin) RNAi phenotype



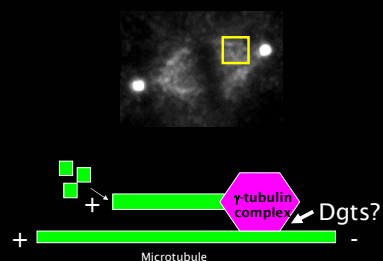
## Dgt (dim $\gamma$ -tubulin) RNAi phenotype



## Dgt (dim $\gamma$ -tubulin) RNAi phenotype

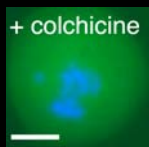
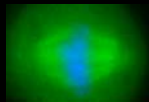


## Are the Dgts Docking Factors for Spindle $\gamma$ -Tubulin?



Are the Dgts Docking Factors for Spindle  $\gamma$ -Tubulin?

Dgt3-GFP



Dgts are in the right spot to be spindle-specific  $\gamma$ -tubulin docking factors

Is spindle localization of  $\gamma$ -tubulin by Dgts important?

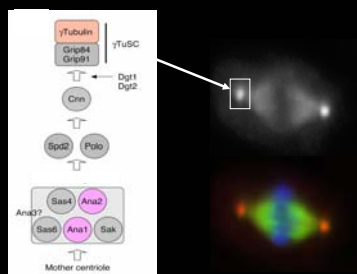
High Throughput Live Cell Imaging to Examine RNAi Phenotypes



Spindle localized  $\gamma$ -tubulin and Dgts help to build kinetochore fibers and align chromosomes

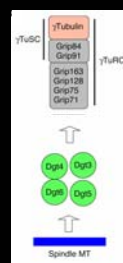
Spindle without Dgt  
(Dgt3 RNAi)

Molecular Model for  $\gamma$ -Tubulin Localization  
(From the RNAi screen and follow-up)



J. Luders, U.K. Patel, T. Stearns *Nature Cell Biol.* (2006)  
Verollet et al. *J. Cell Biol.* (2006)

Molecular Model for  $\gamma$ -Tubulin Localization  
(From the RNAi screen and follow-up)

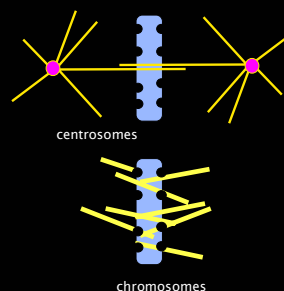




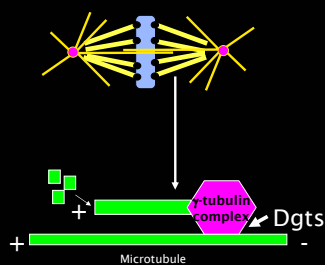
Spindle localized  $\gamma$ -tubulin appears to be more important than the centrosome in *Drosophila*!

(Loss leads to spindle defects/chromosome misalignment)

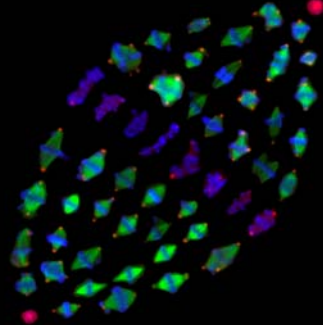
Hypothesis:  
Centrosomes and/or chromatin get the spindle started by nucleating the initial microtubules,



but then the spindle self-propagates making more microtubules by spindle  $\gamma$ -tubulin



Studying the spindle:  
relevance for cancer



Inhibitors of have proved effective as anti-cancer agents by interfering with mitotic spindle function

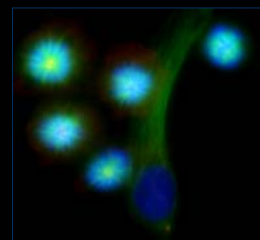
Paclitaxel  
Docetaxel

Vincristine  
Vinblastine  
Vinorelbine

Image from Alexey Khodjakov, Wadsworth Institute

Kinesin 5 (Mitosis Specific) is Essential for Bipolar Spindle Formation

Without Kinesin 5 function- monopolar spindles



Chromosomes - Microtubules - Centrosomes  
(SKOV3)

